<Review>Stereochemical Diversity in Lignan Biosynthesis

AUTHOR(S):

UMEZAWA, Toshiaki; OKUNISHI, Tomoya; SHIMADA, Mikio

CITATION:

UMEZAWA, Toshiaki ...[et al]. <Review>Stereochemical Diversity in Lignan Biosynthesis. Wood research : bulletin of the Wood Research Institute Kyoto University 1997, 84: 62-75

ISSUE DATE:

1997-09-30

URL:

http://hdl.handle.net/2433/53199

Review

Stereochemical Diversity in Lignan Biosynthesis*[1]

Toshiaki UMEZAWA*[2], Tomoya OKUNISHI*[2],
and Mikio SHIMADA*[2]

(Received May 31, 1997)

Abstract——Literature survey of enantiomeric compositions of several lignans isolated from various plants indicated that these plants produce (or accumulate) different enantiomers of the lignans with various enantiomeric compositions. Some are optically pure while the others are mixtures of both enantiomers. The data as well as recent results of enantioselective lignan synthesis with *Forsythia* and *Arctium* enzymes indicated that different stereochemical mechanisms are operating to give rise to the different enantiomers in *Forsythia* spp., *Arctium lappa*, *Wikstroemia* spp., *Phyllanthus* sp., and *Zanthoxylum* spp., and that metabolic steps to produce the optically pure lignans are probably different in the plants. Thus there is a great diversity in stereochemical mechanisms for lignan biosynthesis in different plants.

Keywords: lignan, biosynthesis, stereochemistry, enantiomeric composition, enantiomer

Introduction

During the last decade significant advances have been made in the field of lignan biosynthesis[1–15]. Thus, in 1990 Umezawa *et al.* demonstrated the first example of an enzymatic reaction to produce an optically pure lignan with cell-free extracts of *Forsythia intermedia*[1]. Since then, many reports have been published on enzyme systems involved in lignan biosynthesis with *Forsythia* plants as enzyme sources[2–12]. These studies demonstrated enzymatic formation of the naturally occurring enantiomers of *Forsythia* lignans. On the other hand, there are many examples of plants which produce the opposite enantiomers to those occurring in *Forsythia* spp.[13–18]. Recent studies of stereochemistry of lignan biosynthesis revealed that not only the sign of optical rotation, i.e. predominant enantiomers, of particular lignans, but also enantiomeric composition, i.e. % enantiomer excess values (% e.e.), can vary largely with plant species[18]. The results indicate that there is a great diversity in stereochemical mechanisms of lignan biosynthesis in different plants. The aim of the present review article is to discuss the stereochemical diversity in lignan biosynthesis.

*[1] A part of this review article was presented in 9th International Symposium on Wood and Pulping Chemistry, June 9–12, 1997, Montrél, Canada.

*[2] Laboratory of Biochemical Control, Wood Research Institute, Kyoto University Uji, Kyoto 611, Japan.

Enantiomeric Compositions of Lignans

Absolute configurations of lignans

In order to discuss the stereochemical relationship between various lignans, it is important to correlate the absolute configurations of the lignans. Thus, (+)-enantiomers of the furofuran lignans [pinoresinol **(+)-1**[13,19–23], syringaresinol **(+)-2**[24], eudesmin **(+)-3**[19], sesamin **(+)-4**[19,20]], (+)-enantiomers of the furan lignans [lariciresinol **(+)-5**[13,19–23], dihydrosesamin **(+)-6**[25]], (−)-enantiomers of the dibenzylbutane lignans [secoisolariciresinol **(−)-7**[13,19–23] and phyllanthin **(−)-8**[26]], (−)-enantiomers of dibenzylbutyrolactone lignans [matairesinol **(−)-9**[21–23,27], arctigenin **(−)-10**[21–23,28–30], kusunokinin **(−)-11**[31], traxillagenin **(−)-12**[32], pluviatolide **(−)-13**[33], hinokinin **(−)-14**[21–23,28–30,34,35], haplomyrfolin **(−)-15**[36], thujaplicatin methyl ether **(−)-16**[37,38]], and a hydroxydibenzylbutyrolactone lignan, (−)-wikstromol (=nortrachelogenin) **(−)-17**[39,40], have the same absolute configuration at C8 and C8′ with respect to carbon skeletons. In other words, they can be interconverted with retention of the configuration at C8 and C8′.

Enantiomeric compositions of lignans from various plants

The sign of specific rotation indicates the predominant enantiomer, and the enantiomeric compositions can be estimated from the specific rotation values when the value of optically pure sample is known. However, measurement of specific rotation requires relatively large amounts of samples (more than 5–10 mg), and the measured values sometimes have serious error. Recently, chiral HPLC technique has been developed significantly. By the technique, precise enantiomeric compositions of lignans can be determined with only a few μg of samples, and the technique has been successfully applied to determine precise values of enantiomeric compositions of lignans[41,42].

Table 1 shows a list of enantiomeric compositions (expressed in % e.e.) determined by chiral HPLC analysis or specific rotation ($[\alpha]_D$) of several lignans isolated from various plants. As shown in the Table 1, many lignans have been proved to be optically pure (>99% e.e.), while there are many examples of lignans which are not optically pure, i.e. mixtures of both enantiomers. Thus, all the dibenzylbutyrolactone lignans (including wikstromol **17**) of which enantiomeric compositions have so far been examined by chiral HPLC are found to be optically pure. Recrystallization can alter enantiomeric compositions, but all the dibenzylbutyrolactone lignans applied to chiral columns, except for (−)-arctigenin **(−)-10** from *F. intermedia*[8], were purified chromatographically but not by recrystallization, and, therefore, possible changes of % e.e. values due to recrystallization were eliminated.

In marked contrast, there are no examples of furofuran and furan lignans which are proved to be optically pure by chiral HPLC analysis. Pinoresinol **1** isolated from

Table 1. Specific Rotation and Enantiomeric Compositions of Several Lignans Isolated from Various Plants.

Plant	Lignans and their specific rotation ($[\alpha]_D$) or enantiomeric composition (% e.e.*)				
	Furofuran	Furan	Dibenzylbutane	Dibenzylbutyrolactone	
Thymelaeaceae					
Wikstroemia elliptica	(+)-syringaresinol **(+)-2** +5.9° ($CHCl_3$)[43]	(±)-lariciresinol **(±)-5** −0.2° (acetone)[43]			
Wikstroemia indica				(+)-arctigenin **(+)-10** +28.05° (EtOH)[44]	(+)-nortrachelogenin **(+)-17**** +15.4° ($CHCl_3$)[45] (+)-nortrachelogenin **(+)-17**[46]
Wikstroemia viridiflora (=*indica*)	(+)-pinoresinol **(+)-1** +84.0° (acetone)[47]				(+)-wikstromol **(+)-17** +72° ($CHCl_3$)[47]
Wikstroemia foetida var. *oahuensis*					(+)-wikstromol **(+)-17** +41° ($CHCl_3$)[48]
Wikstroemia sikokiana	(−)-pinoresinol **(−)-1** 74% e.e.[49]	(−)-lariciresinol **(−)-5** 39% e.e.[50]	(−)-secoisolariciresinol **(−)-7** 45% e.e.[50]	(+)-matairesinol **(−)-9** >99% e.e.[49] (+)-kusunokinin **(−)-11** >99% e.e.[50]	(+)-wikstromol **(+)-17** >99% e.e.[49]
Daphne tangutica	(−)-pinoresinol **(−)-1** −34.7° ($CHCl_3$)[51] (±)-syringaresinol **(±)-2** −2.1° ($CHCl_3$)[51]	(−)-lariciresinol **(−)-5** −12.3°(acetone)[51] (−)-dihydrosesamin **(−)-6** −15.9° (pyridine)[51] (−)-dihydrosesamin **(−)-6** −15.9° (pyridine)[25]			
Daphne odora				(+)-matairesinol **(−)-9** +33.7°[52]	(+)-nortrachelogenin **(+)-17** +48°[52]
Passerina vulgaris	(+)-syringaresinol **(+)-2** +13.1° ($CHCl_3$)[53]				(+)-nortrachelogenin **(+)-17** +28.5° ($CHCl_3$)[53]
Dirca occidentalis	(+)-syringaresinol **(+)-2** +12.8° ($CHCl_3$)[54]	(−)-lariciresinol **(−)-5** −17.8° (acetone)[54]			
Stellera chamaejasme	(+)-pinoresinol **(−)-1** +21° (methanol)[55]			(−)-matairesinol **(−)-9** −52° (methanol)[55]	
Oleaceae					
Forsythia koreana	(+)-pinoresinol **(+)-1** >92% e.e.[41]	(+)-lariciresinol **(+)-5**[9]	(−)-secoisolariciresinol **(−)-7** >99% e.e.[41]	(−)-matairesinol **(−)-9** >99% e.e.[41] (−)-arctigenin **(−)-10** >99% e.e.[41]	

Table 1. cont'd.

Plant	Furofuran	Furan	Dibenzylbutane	Dibenzylbutyrolactone
Oleaceae				
Forsythia intermedia			(−)-secoisolariciresinol **(−)-7** >99% e.e.[2]	(−)-matairesinol **(−)-9** >99% e.e.[2] (−)-arctigenin **(−)-10** >99% e.e.[8] (−)-matairesinol **(−)-9** −50.0° (methanol)[56] (−)-arctigenin **(−)-10** −27.5° (methanol)[56] (−)-matairesinol **(−)-9** −41.8° (ethanol)[57] (−)-arctigenin **(−)-10** −34.6° (ethanol)[57]
Forsythia suspensa	(+)-pinoresinol **(+)-1**[3]			
Forsythia spp.	(+)-pinoresinol **(+)-1** +61.6° ($CHCl_3$)[58]			(−)-matairesinol **(−)-9** −41.8° (ethanol)[58]
Fraxinus spp.	(+)-pinoresinol **(+)-1** +77.5° ($CHCl_3$)[59]			
Compositae				
Actium lappa			(+)-secoisolariciresinol **(+)-7**† 81% e.e.[18,60,61]	(−)-matairesinol **(−)-9**† >99% e.e.[18,61] (−)-arctigenin **(−)-10**† >99% e.e.[18,61] (−)-arctigenin **(−)-10** −16.6° ($CHCl_3$)[62] (−)-arctigenin **(−)-10**† −28.69° ($CHCl_3$)[28]
Apocynaceae				
Trachelospermum asiaticum var. *intermedium*				(−)-nortrachelogenin **(−)-17** −16.8° (ethanol)[63] (−)-matairesinol **(−)-9**† −40.0° (ethanol)[64] (−)-arctigenin **(−)-10**† −37.7° (ethanol)[64] (−)-traxillagenin **(−)-12** −25.1° (ethanol)[32]

Table 1. cont'd.

Plant	Furofuran	Furan	Dibenzylbutane	Dibenzylbutyrolactone
Apocynaceae				
Trachelospermum axillare				(−)-traxillagenin **(−)-12**[65] (−)-nortrachelogenin **(−)-17**[65]
Euphorbiaceae				
Phyllanthus niruri			(+)-phyllanthin **(+)-8** +12.42° ($CHCl_3$)[26,66]	
Phyllanthus sp.			(+)-secoisolariciresinol **(+)-7** 98% e.e.[67]	
Rutaceae				
Zanthoxylum pluviatile				(−)-pluviatolide **(−)-13** −35.5° ($CHCl_3$)[68]
Zanthoxylum ailanthoides	(−)-pinoresinol **(−)-1** −42.1° (acetone)[69] (−)-syringaresinol **(−)-2** −9.6° ($CHCl_3$)[69]		(−)-secoisolariciresinol **(−)-7** −46.0° (acetone)[69]	
Zanthoxylum acanthapodium	(±)-eudesmin **(±)-3** 0° ($CHCl_3$)[70] (+)-sesamin **(+)-4** +64.8° ($CHCl_3$)[70] (+)-sesamin **(+)-4**[71]			
Zanthoxylum piperitum	(−)-sesamin **(−)-4** +68.6° ($CHCl_3$)[72]			
Zanthoxylum kellermanii	(−)-pinoresinol **(−)-1**[73]			(−)-matairesinol **(−)-9**[73]
Zanthoxylum fagara	(+)-eudesmin **(+)-3**[73]			
Zanthoxylum valens	(+)-sesamin **(+)-4**[73]			
Zanthoxylum setulosum	(+)-sesamin **(+)-4**[73]			
Pinaceae				
Larix leptolepis	(+)-pinoresinol **(+)-1** +122.0° (methanol)[74] (+)-pinoresinol **(+)-1** 92% e.e.[75]	(+)-lariciresinol **(+)-5** +17.6° (methanol)[74]	(−)-secoisolariciresinol **(−)-7** −20.7° (methanol)[74]	

Table 1. cont'd.

Plant	Furofuran	Furan	Dibenzylbutane	Dibenzylbutyrolactone
Pinaceae				
Larix decidua			(−)-secoisolariciresinol **(−)-7** −35° (acetone)[76]	
Abies sachalinensis		(+)-lariciresinol **(+)-5** +19.5° (methanol)[77]		
Picea excelsa (=*abies*)				(−)-matairesinol **(−)-9** −45.0° (acetone)[78]
Tsuga mertensiana				(−)-matairesinol **(−)-9** −45.0° (acetone)[79]
Cupressaceae				
Chamaecyparis obtusa				(−)-hinokinin **(−)-14** >99% e.e.[80]
Chamaecyparis obtusa cv. Breviramea				(−)-haplomyrforin **(−)-15** >99% e.e.[80] (−)-pulviatoride **(−)-13** >99% e.e.[80]
Thuja occidentalis				(−)-matairesinol **(−)-9** >99% e.e.[81] (−)-thuyaplicatin methyl ether **(−)-16** >99% e.e.[81]
Araucariaceae				
Araucaria angustifolia		(+)-lariciresinol **(+)-5** +18° (acetone)[82]	(−)-secoisolariciresinol **(−)-7** −32° (acetone)[82]	
Podocarpaceae				
Podocarpus spicatus			(−)-secoisolariciresinol **(−)-7** −35.6°[83]	

*: Enantiomeric compositions expressed in % e.e. were obtained by chiral HPLC. **: Nortrachelogenin=wikstromol. †: Obtained as aglycone after hydrolysis.

Wikstroemia sikokiana was found to be a mixture of both enantiomers in favor of (−)-enantiomer **(−)-1** (74% e.e.)[49], and that from *Larix leptolepis* is dextrorotatory with 92% e.e.[75]. In the case of pinoresinol **1** and lariciresinol **5** isolated from *Forsythia* plants[3,9,41],

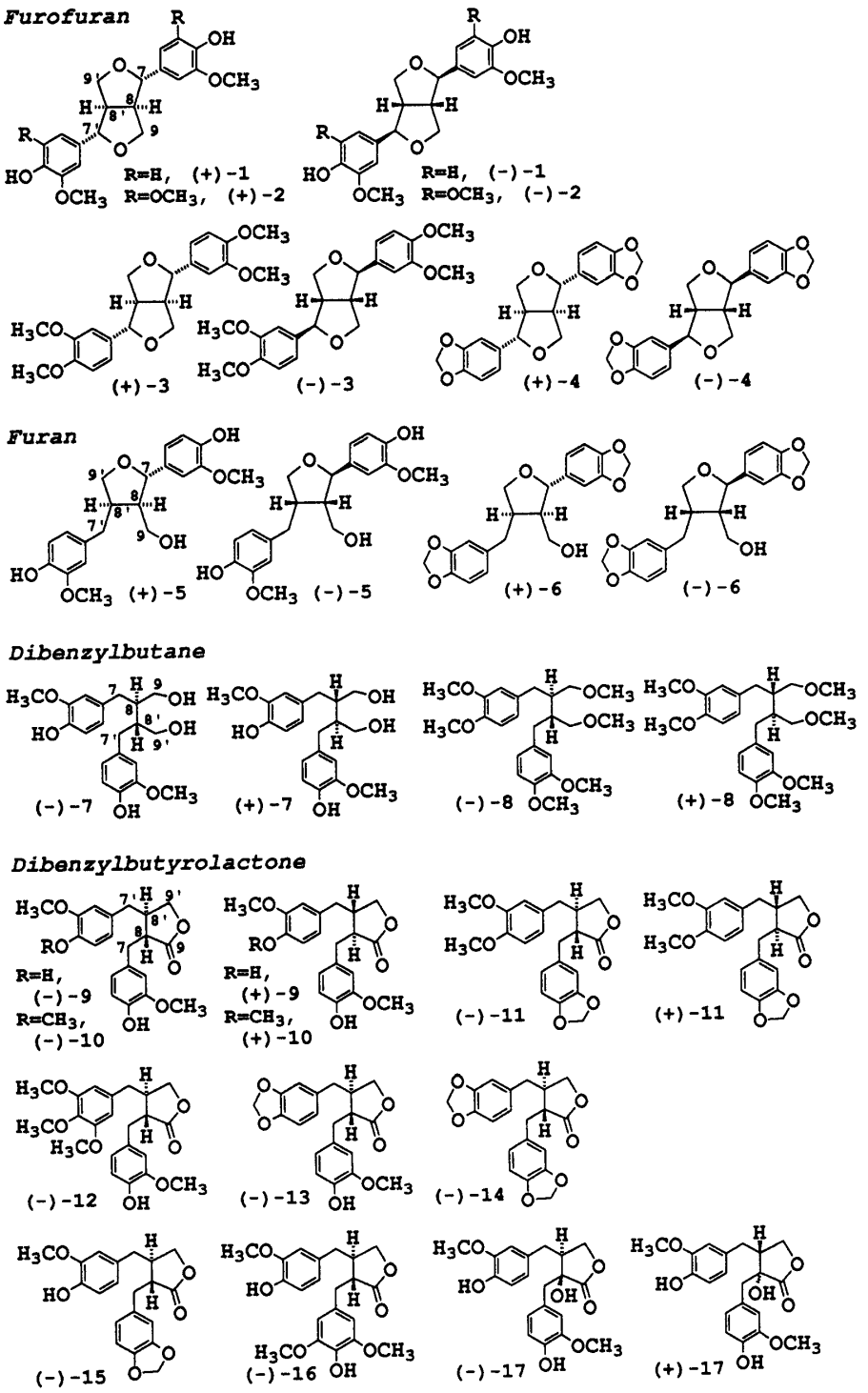

Fig. 1. Structures of lignans.

the peaks of the (+)-enantiomers **(+)-1** and **(+)-5** were accompanied by small peaks which have the same retention volumes as those of (−)-pinoresinol **(−)-1** and (−)-lariciresinol **(−)-5** in the chiral HPLC chromatograms, although the small peaks remain to be identified spectrometrically.

As for dibenzylbutane lignans, both optically pure secoisolariciresinol **7** and that composed of both enantiomers are known: secoisolariciresinol **7** from *Forsythia* plants are optically pure and levorotatory[2,41], and that from *Phyllanthus* sp. are almost optically pure (98% e.e.) in favor of (+)-enantiomer **(+)-7**[67]. On the other hand, secoisolariciresinol **7** isolated from *Wikstroemia sikokiana* and *Arctium lappa* are not optically pure, with 45% e.e., (−)>(+)[50], and 81% e.e., (+)>(−)[18,60,61], respectively.

These stereochemical properties of lignans are summarized in Table 2a and b.

Predominant enantiomers of lignans from various plants

It is also apparent from Table 1 that predominant enantiomers of particular lignans are different among different plant species (Table 2c), and even racemic lignans sometimes occur. Thus, regarding dibenzylbutyrolactone lignans including wikstromol **17**, all the lignans of this class isolated from Thymelaeaceae plants are dextrorotatory, except for (−)-matairesinol **(−)-9** from *Stellera chamaejasme*[55]. On the other hand, this class of lignans obtained from the other plants in the Table 1 are levorotatory.

Similarly, (−)-secoisolariciresinol **(−)-7** was isolated from many plants, e.g. *Wikstroemia sikokiana* (45% e.e.)[50], *Forsythia* plants (>99% e.e.)[2,41], *Zanthoxylum ailanthoides*[69], *Larix leptorepis*[74], *Larix decidua*[76], *Araucaria angustifolia*[82], and *Podocarpus spicatus*[83], while (+)-secoisolariciresinol **(+)-7** was obtained from *Arctium lappa* (81% e.e.)[18,60,61] and *Phyllanthus* sp. (98% e.e.)[67].

Based on the data, it is of interest to compare the absolute configurations of the lignans occurring in each plant species (Table 2d). *Forsythia* plants produce (+)-pinoresinol **(+)-1**[3,41,58], (+)-lariciresinol **(+)-5**[9], (−)-secoisolariciresinol **(−)-7**[2,41], (−)-matairesinol **(−)-9**[2,41,56–58], and (−)-arctigenin **(−)-10**[8,41,56,57] which have the same absolute configurations at C8 and C8′. On the other hand, (+)-arctigenin **(+)-10**[44] and (+)-pinoresinol **(+)-1**[47] isolated from *Wikstroemia indica* (=*viridiflora*) have the opposite absolute configuration at C8 and C8′ each other. Similarly, *Wikstroemia sikokiana* produces (+)-

Table 2. Stereochemical Properties of Lignans from Various Plants.

a) Both optically pure lignans and lignans composed of both enantiomers are known.
b) All the dibenzylbutyrolactone lignans of which enantiomeric compositions have so far been examined by chiral HPLC are opically pure.
c) Predominant enantiomers of a particular lignan can vary with plant sources.
d) Absolute configurations of the predominant enantiomers of lignans occurring in a plant species are often different each other.

matairesinol **(+)-9** (>99% e.e.)[49] and (−)-secoisolariciresinol **(−)-7** (45% e.e.)[50], and the predominant enantiomers of the lignans have the opposite absolute configurations each other, while *Arctium lappa* produce (−)-matairesinol **(−)-9** (>99% e.e.)[18,61] and (+)-secoisolariciresinol **(+)-7** (81% e.e.)[18,60,61]. Another example is the lignans of *Zanthoxylum* spp. (−)-Matairesinol **(−)-9**[73] and (−)-secoisolariciresinol **(−)-7**[69] were isolated from the plants, whereas (−)-pinoresinol **(−)-1** which has the opposite absolute configuration to those of **(−)-9** and **(−)-7** was obtained from the plants[69,73].

The apparent inconsistency, i.e. the fact that absolute configurations of the predominant enantiomers of lignans occurring in a single plant species are often different (Table 2d), is of interest from the view point of stereochemical mechanisms in lignan biosynthesis.

Stereochemistry of Lignan Biosynthesis

Stereochemistry of enzymatic lignan synthesis

Much of the knowledge of enzymatic lignan synthesis has been obtained with *Forsythia* spp. as enzyme sources[1–12], and conversion of coniferyl alcohol **18** to the natural enantiomers of the *Forsythia* lignans by the *Forsythia* enzyme preparations was established as shown in Fig. 2A. Each conversion, except for the final methylation, is well-controlled

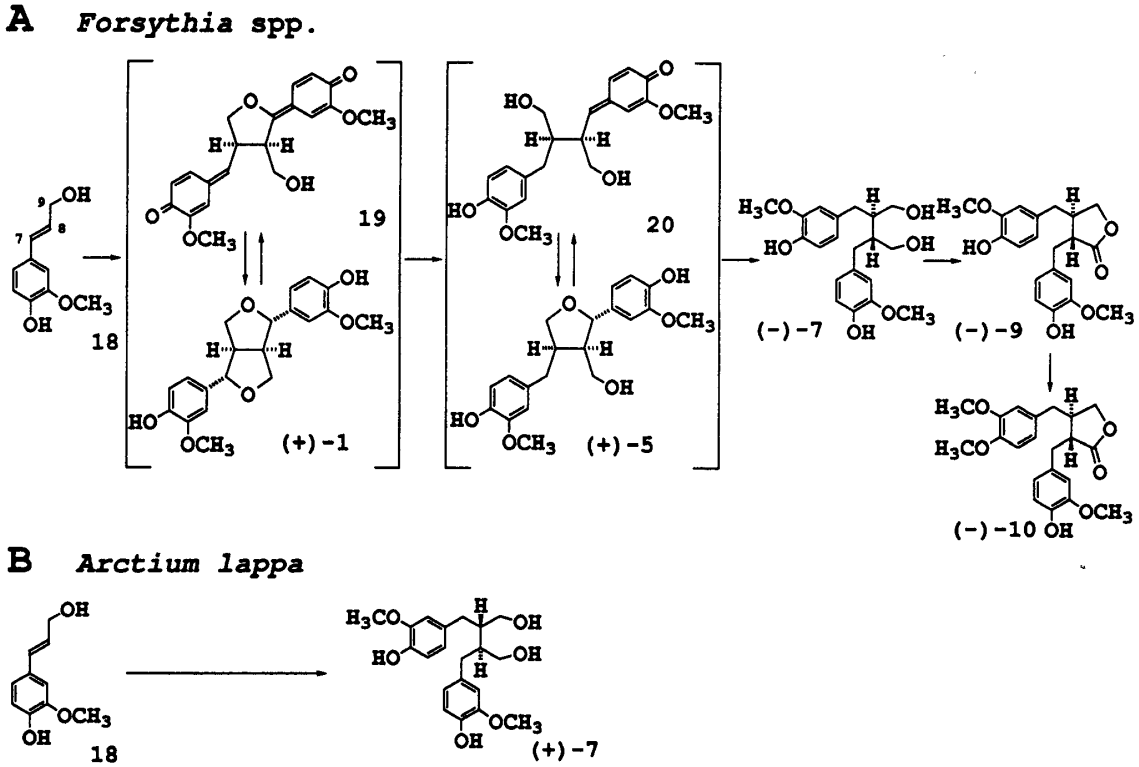

Fig. 2. Enzymatic formation of lignans. A, *Forsythia* spp.; B, *Arctium lappa*.

stereochemically. Very recently, Davin *et al.* found a protein lacking a catalytically active center from *Forsythia* sp[12]. The protein was found to allow enantioselective coupling of two coniferyl alcohol molecules in the presence of oxidative enzymes such as laccase. They coined the word "dirigent protein" for the unique protein. In the subsequent post-coupling steps, (+)-pinoresinol **(+)-1**, (+)-lariciresinol **(+)-5**, and (−)-secoisolariciresinol **(−)-7** are transformed preferentially over the antipodes into almost optically pure **(+)-5**, optically pure **(−)-7**, and optically pure (−)-matairesinol **(−)-9**, respectively, with *Forsythia* enzymes[7,9]. Conversion of secoisolariciresinol **7** to matairesinol **9** with a *Forsythia* enzyme preparation is also controlled stereochemically; only (−)-secoisolariciresinol **(−)-7** was found to be converted to (−)-matairesinol **(−)-9**, but (+)-secoisolariciresinol **(+)-7** was not oxidized to either (+)-matairesinol **(+)-9** or **(−)-9**[2].

On the other hand, enantioselective formation of the other enantiomer (+)-secoisolariciresinol **(+)-7** (20% e.e.) from coniferyl alcohol **18** was demonstrated with *Arctium lappa* enzyme preparation (Fig. 2B)[60]. The predominant enantiomer is the same as that isolated from the plant, but is the opposite antipode to the one occurring in *Forsythia* spp., although the enantiomer excess (20% e.e.) was much smaller than that (81% e.e.) of **(+)-7** isolated from *Arctium lappa*.

Stereochemical diversity in lignan biosynthesis

Analysis of the enantiomeric compositions of the lignans (Table 1) as well as the enantioselective formation of lignans with enzyme preparations indicated that stereochemical control mechanisms involved in lignan biosynthesis of *Wikstroemia* spp., *Arctium lappa*, *Phyllanthus* spp., and *Zanthoxylum* spp. are different from that of *Forsythia* spp. in the following aspects.

First, these plants produce or accumulate different enantiomers of lignans to those occurring in *Forsythia* spp. as summarized in Table 1. Obviously the stereochemical mechanisms to produce different enantiomers are different. Enantioselective formation of (−)- and (+)-secoisolariciresinols **(−)-7** and **(+)-7** has been demonstrated with *Forsythia* spp.[1,5–7,9] and *Arctium lappa*[60] enzymes, respectively (Fig. 2A and B).

Second is regarding the difference in the metabolic steps to produce optically pure lignans. Studies with *Forsythia* enzyme preparations established the enzymatic conversion from coniferyl alcohol **18** to *Forsythia* lignans[1–12] as shown in Fig. 2A. The initial coupling of two coniferyl alcohol molecules are highly enantioselective and almost optically pure (+)-pinoresinol **(+)-1** was formed[10,12]. On the other hand, in *Wikstroemia sikokiana* the initial coupling is not enough enantioselective to produce optically pure lignans[49,50,84]. Feeding experiments with *Wikstroemia sikokiana* strongly suggested the conversion from coniferyl alcohol **18** to matairesinol **9** via secoisolariciresinol **7** (Fig. 3) in the plant, which is a similar metabolic sequence to that in *Forsythia* spp. (Fig. 2A). Since (−)-pinoresinol **(−)-1**, (−)-

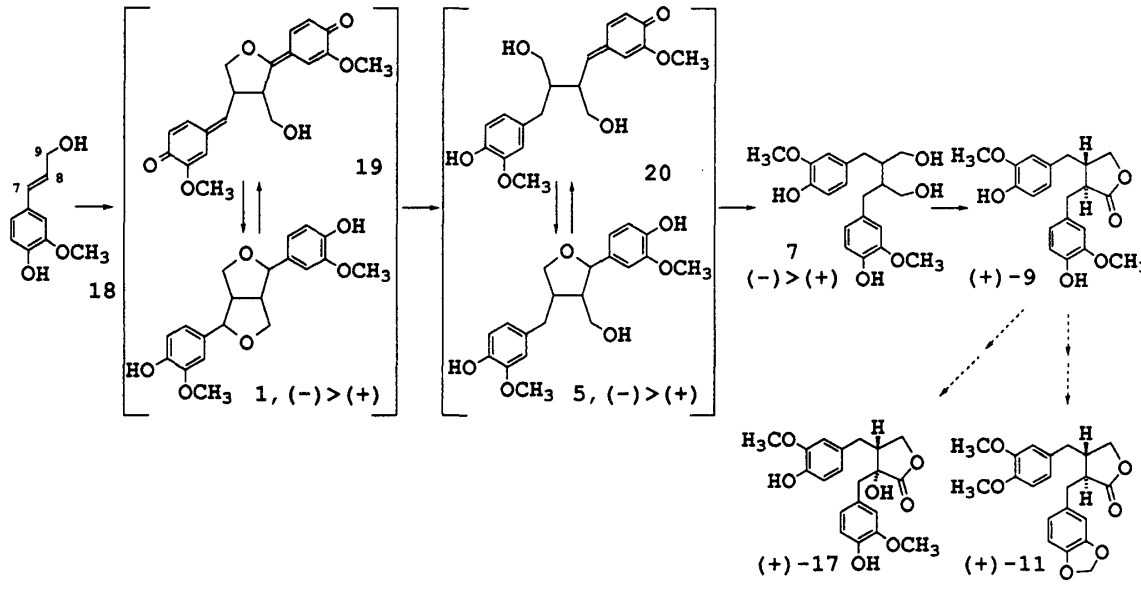

Fig. 3 A possible biosynthetic pathway of *Wikstroemia sikokiana* lignans.

lariciresinol **(−)-5** and (−)-secoisolariciresinol **(−)-7** isolated from *W. sikokiana* are not optically pure and not racemic, not only the initial formation of pinoresinol **1** (or the corresponding quinone methides, e.g. **19**) from coniferyl alcohol **18** but also the post-coupling processes are probably involved in selective formation of one of the enantiomers. In addition, it is strongly suggested that the oxidation of secoisolariciresinol **7** to matairesinol **9** is the key metabolic step to produce the optically pure lignans in this plant. This is in sharp contrast to the lignan biosynthesis in *Forsythia* plants[10,12] (Fig. 2).

Similarly it is strongly suggested that the formation of the dibenzylbutyrolactone lignan is the key metabolic step to produce the optically pure lignans in *Arctium lappa*, since (−)-matairesinol **(−)-9** and (+)-secoisolariciresinol **(+)-7** isolated from this plant are optically pure and not optically pure with 81% e.e., respectively[18,60,61]. Selective conversion of (−)-secoisolariciresinol **(−)-7** to (−)-matairesinol **(−)-9** resulting in accumulation of (+)-secoisolariciresinol **(+)-7** can explain at least partly the enantiomeric composition of secoisolariciresinol **7** in the plant, while enantioselective formation of (+)-secoisolariciresinol **(+)-7** (20% e.e.) from achiral coniferyl alcohol **18** which was demonstrated with cell-free extracts of the plant[60] can also account for the composition in favor of (+)-enantiomer **(+)-7**.

Thus, different stereochemical mechanisms are operating to give rise to the different enantiomers in the plants of the five genera, *Forsythia*, *Arctium*, *Phyllanthus*, *Wikstroemia*, and *Zanthoxylum*, and metabolic steps to produce the optically pure lignans are probably different in the plants. As for the other plants, no data have been obtained for stereochemical mechanisms of lignan biosynthesis, and even precise enantiomeric compositions are not

reported except for *Chamaecyparis*[80], *Thuja*[81], and *Larix*[75] lignans. However, since the stereochemical mechanisms in the five genera are different each other, there may be a great diversity in stereochemical mechanisms of lignan biosynthesis in various plant species.

References

1) T. UMEZAWA, L.B. DAVIN and N.G. LEWIS: *Biochem. Biophys. Res. Commun.*, **171**, 1008–1014 (1990).
2) T. UMEZAWA, L.B. DAVIN and N.G. LEWIS: *J. Biol. Chem.*, **266**, 10210–10217 (1991).
3) T. UMEZAWA, L.B. DAVIN, E. YAMAMOTO, D.G.I. KINGSTON and N.G. LEWIS: *J. Chem. Soc. Chem. Commun.*, **1990**, 1405–1408.
4) L.B. Davin, D.L. Bedgar, T. Katayama and N.G. LEWIS: *Phytochem.*, **31**, 3869–3874 (1992).
5) T. KATAYAMA, L.B. DAVIN and N.G. LEWIS: *Phytochem.*, **31**, 3875–3881 (1992).
6) T. KATAYAMA, L.B. DAVIN, A. CHU and N.G. LEWIS: *Phytochem.*, **33**, 581–591 (1993).
7) A. CHU, A. DINKOVA, L.B. DAVIN, D.L. BEDGAR and N.G. LEWIS: *J. Biol. Chem.*, **268**, 27026–27033 (1993).
8) S. OZAWA, L.B. DAVIN and N.G. LEWIS: *Phytochem.*, **32**, 643–652 (1993).
9) T. UMEZAWA, H. KURODA, T. ISOHATA, T. HIGUCHI and M. SHIMADA: *Biosci. Biotech. Biochem.*, **58**, 230–234 (1994).
10) P.W. PARÉ H.-B. WANG, L.B. DAVIN and N.G. LEWIS: *Tetra. Lett.*, **35**, 4731–4734 (1994).
11) A.T. DINKOVA-KOSTOVA, D.R. GANG, L.B. DAVIN, D.L. BEDGAR, A. CHU and N.G. LEWIS: *J. Biol. Chem.*, **271**, 29473–29482 (1996).
12) L.B. DAVIN, H.-B. WANG, A.L. CROWELL, D.L. BEDGAR, D.M. MARTIN, S. SARKANEN and N.G. LEWIS: *Science*, **275**, 362–366 (1997).
13) D.C. AYRES and J.D. LOIKE: "Lignans Chemical, Biological and Clinical Properties", Cambridge University Press, Cambridge (1990).
14) T. UMEZAWA: *Mokuzai Gakkaishi*, **42**, 911–920 (1996).
15) T. UMEZAWA: "Biochemistry and Molecular Biology of Wood", T. Higuchi, Springer-Verlag, Berlin, p. 181–194 (1997).
16) C.B.S. RAO: "Chemistry of Lignans", Andhra University Press, Andhra Paradesh (1978).
17) O.R. GOTTLIEB and M. YOSHIDA: "Natural products of woody plants", J.W. Rowe, ed., Springer-Verlag, Berlin, p. 439–511 (1989).
18) T. UMEZAWA, T. OKUNISHI and M. SHIMADA: "ACS Symp. Ser.", N.G. Lewis and S. Sarkanen, eds., American Chemical Society, Washington, D.C., in press.
19) A. PELTER and R.S. WARD: "Chemistry of Lignans", C.B.S. Rao, ed., Andhra University Press, Andhra Paradesh, India p. 227–275 (1978).
20) K. FREUDENBERG and G.S. SIDHU: *Tetra. Lett.*, **No. 20**, 3–6 (1960).
21) R. STEVENSON: "Chemistry of Lignans", C.B.S. Rao, ed., Andhra University Press, Andhra Paradesh, India, p. 65–94 (1978).
22) Y. KATO and K. MUNAKATA: "Chemistry of Lignans", C.B.S. Rao, ed., Andhra University Press, Andhra Paradesh, India, p. 95–122 (1978).
23) K. WEINGES and R. SPÄNIG: "Oxidative coupling of phenols", W.I. Taylor and A.R. Battersby, eds., Marcel Dekker, p. 323–355 (1967).
24) I.R. BYTHEWAY, E.L. GHISALBERTI, S. GOTSIS, P.R. JEFFERIES, B.W. SKELTON, K.E. SUGARS and A.H. WHITE: *Aust. J. Chem.*, **40**, 1913–1917 (1987).
25) L.-G. ZHUANG, O. SELIGMANN, H. LOTTER and H. WAGNER: *Phytochem.*, **22**, 265–267 (1983).
26) L.R. ROW, P. SATYANARAYANA and G.S.R. SUBBA RAO: *Tetrahedron*, **23**, 1915–1918 (1967).
27) A.W. SCHRECKER and J.H. HARTWELL: *J. Am. Chem. Soc.*, **79**, 3827–3831 (1957).
28) T. OMAKI: *Yakugaku Zasshi*, **55**, 816–827 (1935).
29) T. OMAKI: *Yakugaku Zasshi*, **56**, 982–985 (1936).
30) R.D. HAWORTH and T. RICHARDSON: *J. Chem. Soc.*, **1935**, 633–636.

31) E. Brown and A. Daugan: *Tetra. Lett.*, **27**, 3719–3722 (1986).
32) S. Nishibe, K. Okabe and S. Hisada: *Chem. Pharm. Bull.*, **29**, 2078–2082 (1981).
33) S. Nishibe, M. Chiba and S. Hisada: *Yakugaku Zasshi*, **97**, 1366–1369 (1977).
34) S. Keimatsu and T. Ishiguro: *Yakugaku Zasshi*, **56**, 399–404 (1936).
35) H. Yoda, S. Naito, K. Takabe, N. Tanaka and K. Hosoya: *Tetra. Lett.*, **31**, 7623–7626 (1990).
36) U. Evcim, B. Gözler, A.J. Freyer and M. Shamma: *Phytochem.*, **25**, 1949–1951 (1986).
37) S. Nishibe, S. Hisada and I. Inagaki: *Yakugaku Zasshi*, **94**, 522–524 (1974).
38) E. Brown and A. Daugan: *Heterocycles*, **26**, 1169–1172 (1987).
39) K. Khamlach, R. Dhal and E. Brown: *Tetra. Lett.*, **30**, 2221–2224 (1989).
40) J.L. Belletire, D.M. Ho and D.F. Fry: *J. Nat. Prod.*, **53**, 1587–1592 (1990).
41) T. Umezawa, T. Isohata, H. Kuroda, T. Higuchi and M. Shimada: "Biotechnology in Pulp and Paper Industry", M. Kuwahara and M. Shimada, eds., Uni Publ., Tokyo, p. 507–512 (1992).
42) L.B. Davin, T. Umezawa and N.G. Lewis: "Modern Phytochemical Methods, Recent Advances in Phytochemistry, Vol. 25, N.H. Fischer, M.B. Isman and H.A. Stafford, eds., Plenum, New York, p. 75–112 (1991).
43) C.-Y. Duh, C.H. Phoebe, Jr., J.M. Pezzuto, A.D. Kinghorn and N.R. Farnsworth: *J. Nat. Prod.*, **49**, 706–709 (1986).
44) H. Suzuki, K.-H. Lee, M. Haruna, T. Iida, K. Ito and H.-C. Huang,: *Phytochem.*, **21**, 1824–1825 (1982).
45) A. Kato, Y. Hashimoto and M. Kidokoro: *J. Nat. Prod.*, **42**, 159–162 (1979).
46) K.-H. Lee, K. Tagahara, H. Suzuki, R.-Y. Wu, M. Haruna, I.H. Hall, H.-C. Huang, K. Ito, T. Iida, and J.-S. Lai: *J. Nat. Prod.*, **44**, 530–535 (1981).
47) S. Tandon and R.P. Rastogi: *Phytochem.*, **15**, 1789–1791 (1976).
48) S.J. Torrance, J.J. Hoffmann and J.R. Cole: *J. Parm. Sci.*, **68**, 664–665 (1979).
49) T. Umezawa and M. Shimada: *Mokuzai Gakkaishi*, **42**, 180–185 (1996).
50) T. Okunishi, T. Umezawa and M. Shimada: *Mokuzai Gakkaishi*, submitted.
51) L.-g. Zhuang, O. Seligmann, K. Jurgic and H. Wagner: *Planta Med.*, **45**, 172–176 (1982).
52) S. Kogiso, K. Wada and K. Munakata: Report of the 26th International Congress of Pure and Applied Chemistry, Tokyo, p. 291 (1977).
53) J.-X. Guo, S.S. Handa, J.M. Pezzuto, A.D. Kinghorn and N.R. Farnsworth: *Planta Med.*, **50**, 264–265 (1984).
54) M.M. Badawi, S.S. Handa, A.D. Kinghorn, G.A. Cordell and N.R. Farnsworth: *J. Pharm. Sci.*, **72**, 1285–1287 (1983).
55) H. Tatematsu, M. Kurokawa, M. Niwa and Y. Hirata: *Chem. Pharm. Bull.*, **32**, 1612–1613 (1984).
56) M.M.A. Rahman, P.M. Dewick, D.E. Jackson and J.A. Lucas: *Phytochem.*, **29**, 1971–1980 (1990).
57) S. Nishibe, A. Sakushima, S. Kitagawa, B. Klimek, R. Benecke and H. Thieme: *Shoyakugaku Zasshi*, **42**, 324–328 (1988).
58) S. Kitagawa, S. Nishibe, R. Benecke and H. Thieme: *Chem. Pharm. Bull.*, **36**, 3667–3670 (1988).
59) H. Tsukamoto, S. Hisada and S. Nishibe: *Chem. Pharm. Bull.*, **32**, 4482–4489 (1984).
60) T. Umezawa and M. Shimada: *Biosci. Biotech. Biochem.*, **60**, 736–737 (1996).
61) T. Umezawa and M. Shimada: *Biosci. Biotech. Biochem.*, submitted.
62) B.H. Han, Y.H. Kang, H.O. Yang and M.K. Park: *Phytochem.*, **37**, 1161–1163 (1994).
63) S. Nishibe, S. Hisada and I. Inagaki: *Phytochem.*, **10**, 2231–2232 (1971).
64) I. Inagaki, S. Hisada and S. Nishibe: *Chem. Pharm. Bull.*, **20**, 2710–2718 (1972).
65) S. Nishibe, T. Fujimoto, M. Nose, T. Takeda, Y. Ogihara and G. Xu: *Phytochem.*, **32**, 1579–1581 (1993).
66) L.R. Row, C. Srinivasulu, M. Smith and G.S.R. Subba Rao: *Tetra. Lett.*, 1557–1567 (1964).
67) T. Okunishi, T. Umezawa and M. Shimada: unpublished.
68) J.E.T. Corrie, G.H. Green, E. Ritchie and W.C. Taylor: *Aust. J. Chem.*, **23**, 133–145 (1970).
69) H. Ishii, T. Ishikawa, M. Mihara and M. Akaike: *Yakugaku Zasshi*, **103**, 279–292 (1983).
70) E. Venkata Rao, K.V. Sasthry and T.J. Palanivelu: *Curr. Sci.*, **44**, 228-230 (1975).

71) A. PERTER, R.D. WARD, E. VENKATA RAO and K.V. SASTRY: *Tetrahedron*, **32**, 2783–2788 (1976).
72) F. ABE, S. YAHARA, K. KUBO, G. NONAKA, H. OKABE and I. NISHIOKA: *Chem. Pharm. Bull.*, **22**, 2650–2655 (1974).
73) M. MARCOS, C. JIMÉNEZ, M.C. VILLAVERDE, R. RIGUERA, L. CASTEDO and F. STERMITZ: *Planta Med.*, **56**, 89–91 (1990).
74) K. MIKI, K. ITO and T. SASAYA: *Mokuzai Gakkaishi*, **25**, 665–670 (1979).
75) K. NABETA, K. NAKAHARA, J. YONEKUBO, H. OKUYAMA and T. SASAYA: *Phytochem.*, **30**, 3591–3593 (1991).
76) K. FREUDENBERG and K. WEINGES.: *Tetra. Lett.*, **No. 17**, 19–22 (1959).
77) T. SASAYA, T. TAKEHARA and T. KOBAYASHI: *Mokuzai Gakkaishi*, **26**, 759–764 (1980).
78) K. FREUDENBERG and L. KNOF: *Ber.*, **90**, 2857–2869 (1957).
79) G.M. BARTON and J.A.F. GARDNER: *J. Org. Chem.*, **27**, 322–323 (1962).
80) D.H. CHOI, T. UMEZAWA and M. SHIMADA: unpublished.
81) S. KAWAI, K. SUGISHITA and H. OHASHI: Abst. 9th International Symposium on Wood and Pulping Chemistry, Montréal, p. 46 (1997).
82) S.F. FONSECA, J. DE PAIVA CAMPELLO, L.E.S. BARATA and E.A. RÚVEDA: *Phytochem.*, **17**, 499–502 (1978).
83) L.H. BRIGGS, R.C. CAMBIE and J.L. HOARE: *Tetra. Lett.*, **No. 4**, 14–15 (1959).
84) T. OKUNISHI, T. UMEZAWA and M. SHIMADA: *Wood Research*, **No. 84**, 25–27 (1997).